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(54) Title: CONFORMATIONALLY CONSTRAINED LABELED PEPTIDES FOR IMAGING AND THERAPY

(57) Abstract: Conformational constraints in diagnostic and therapeutic agents in peptides have been introduced by utilization of disulfide bonds and amide cyclizations. These constraints are responsible for altering the stability and specificity of these receptor-targeted agents. Conformationally constrained peptides containing secondary and primary amines, ethers, thioethers, amidines, esters and other functionalities have been synthesized. Methods are disclosed which incorporate multiple features of the above functionalities in the macrocyclic ring of the peptides.

TITLE OF THE INVENTION

# CONFORMATIONALLY CONSTRAINED LABELED PEPTIDES FOR IMAGING AND THERAPY

## FIELD OF INVENTION

The present invention relates to conformationally constrained receptor targeted radiolabeled peptides which are amenable to positioning of a chelating moiety (henceforth referred to as "CM") and/or diagnostic or therapeutic isotopes. The methodology is applicable to several families of peptides including but not limited to: somatostatin, gastrin, gastrin releasing peptide, bombesin and bombesin antagonists, gastrin releasing peptides, adhesion peptides, cholecystokinin, neurotensins, neuropeptide Y, vasoactive intestinal peptides, thyroid stimulating hormone, angiotensin, pancreatic adenylate cyclase activating peptide, and substance P. Instead of chelating moieties containing diagnostic and therapeutic isotopes, other diagnostic agents such as fluorescent dyes, dyes that absorb at the near infrared region, can be attached at the same position. Also, instead of chelating moieties, other therapeutic agents such as physiologically acceptable drugs can be attached at the same position.

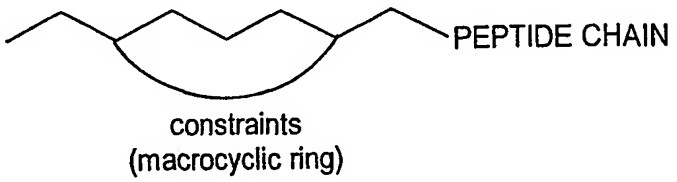
## BACKGROUND OF THE INVENTION

Over the years, the presence of various receptors has been demonstrated in a wide variety of tumors. Diagnostic agents based on peptides have been introduced. In-111-DTPA-somatostatin analogs (see U.S. Patents 5,753,627 and 5,776,894) were introduced for the purpose of imaging and therapy of somatostatin subtype-2 receptors. In this case, all the chelating moieties were attached to the N-terminus of the peptides. The proteins or antibodies were either radioiodinated or reacted with bifunctional chelating agents and randomly substituted.

## SUMMARY OF THE INVENTION

Conformational constraints in diagnostic and therapeutic agents in peptides have been introduced by means of disulfide bonds and amide cyclizations. These constraints are responsible for altering the stability and specificity of these receptor-targeted agents.

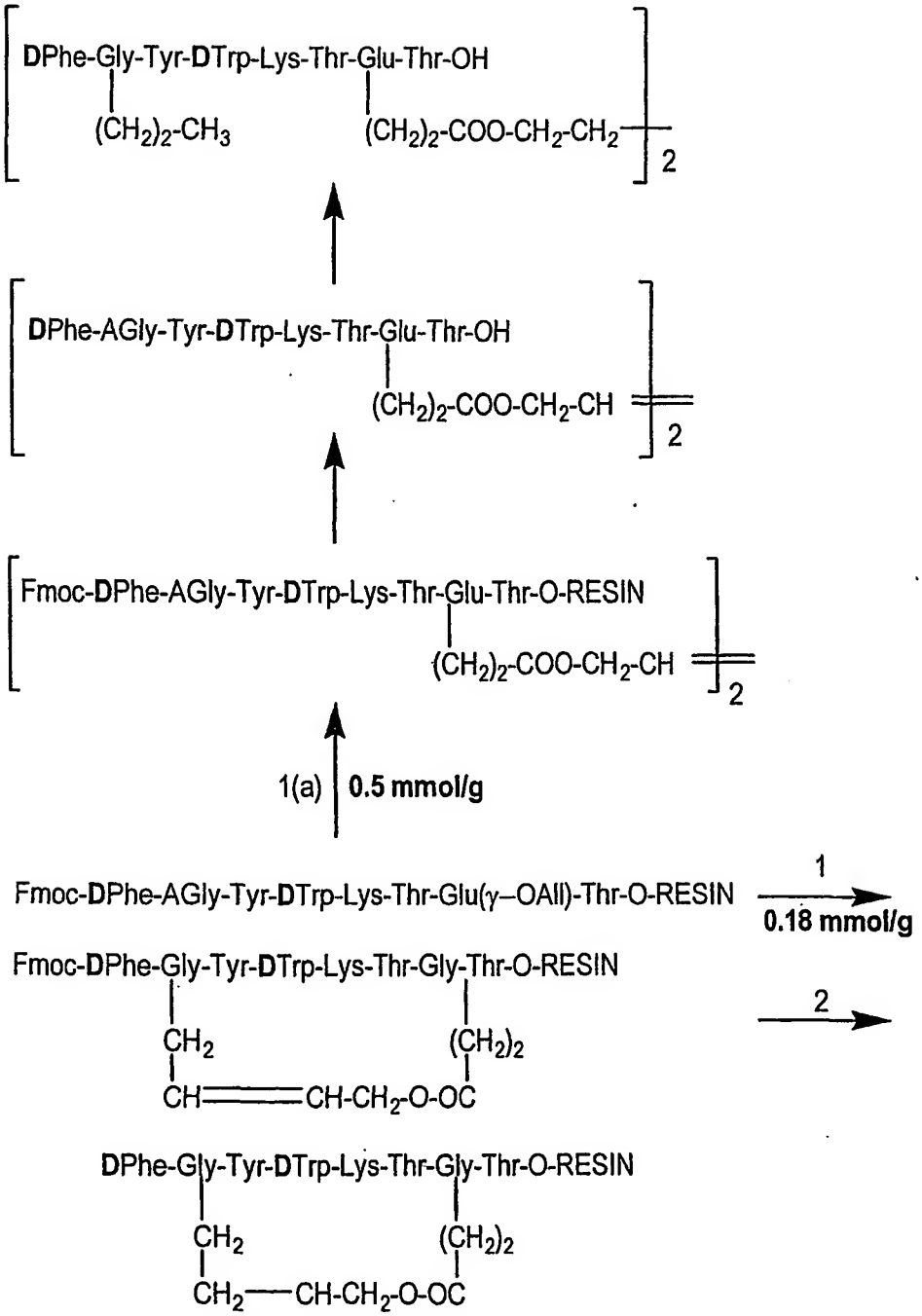
Conformationally constrained peptides containing secondary and primary amines, ethers, thioethers, amidines, esters and other functionalities have been synthesized. Methods are disclosed which provide means for incorporating multiple features of the above functionalities in the macrocyclic ring of the peptides.




In many instances, there is a specific need for attachment of the chelating moieties away from the binding sites (besides the N-terminus, C-terminus and side chains of the amino acid sequences). Incorporation of amines in the macrocyclic ring provides a handle for the incorporation of the chelating moiety away from the binding sites. Incorporation of ether and thioether and other functionalities allows isosteric substitution of the macrocyclic ring. Incorporation of esters in the macrocyclic ring provides stability to the ring and a means towards rapid degradation and elimination after localization in the excretionary organs. It is understood that a combination of the above features can be incorporated between any two positions of the amino acid chain of the peptide. In addition to or in place of attaching chelating moieties, other moieties may be attached to the macrocyclic ring. Such other moieties include, but are not limited to, dyes which are useful for detection such as for diagnostic purposes and drugs which can be used for therapeutic purposes.

## DETAILED DESCRIPTION OF THE INVENTION

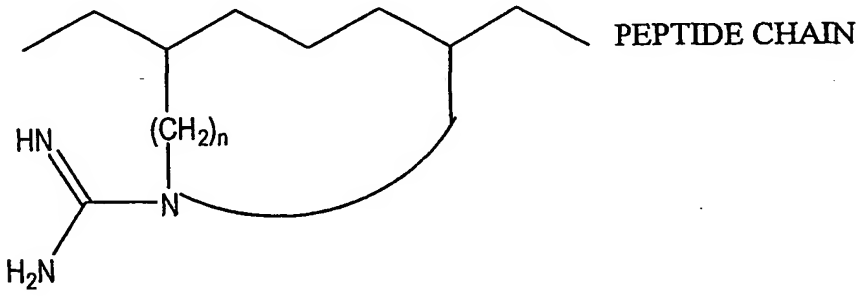
It is well known in the field of peptide chemistry that cyclization of peptides alters stability and specificity of the peptides. The conformation of a peptide can be stabilized or fixed by the introduction of a ring. In several naturally occurring peptides, the conformation is stabilized by the presence of disulfide or lactam bridges. Peptides containing disulfide bridges undergo metabolism with the formation of cysteines followed by enzymatic degradation of the peptide. Isosteric substitution of the disulfide bridge with either $CH_2$-S or $CH_2$-$CH_2$ bridge should not only inhibit metabolism of the peptide, but also prolong the serum half-life of the peptide. Such a modification, however, may also render rigidity to the ring resulting in an inactive compound.




Metathesis reaction of linear bombesin and neurotensin derivatives resulted in cyclic esters.

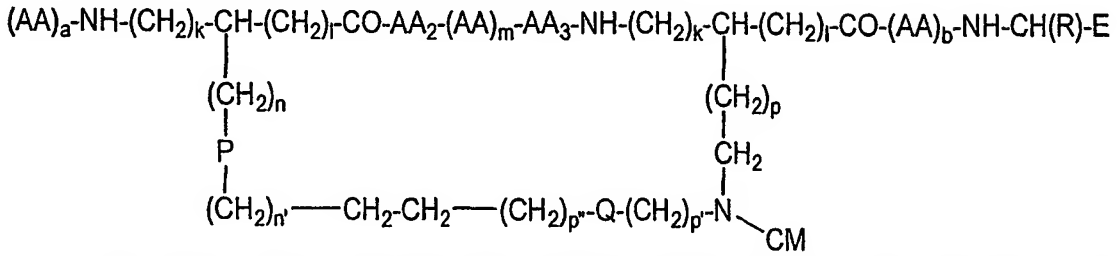
We have developed a method of stabilizing arginines by using amidine nitrogen to provide stabilization and to provide specificity. This method can be used for any arginine containing peptide, including those with an Arg-Gly-Asp (RGD) sequence. Metathesis

reactions can be performed on RGD containing peptides. RGD containing peptides have been implicated as inhibitors of integrin-ligand interaction in studies of cell adhesion, migration and differentiation. In the present literature, all of the Arg-Gly-Asp peptides are either linear or the sequence is contained within a cyclic structure to provide stability and specificity. The methods disclosed herein use the Arg amidine nitrogen to stabilize the conformation of the RGD molecules. This arrangement results in stability against enzymatic degradation.




The discussion above, together with the specific examples discussed below, show that a metal complex-catalyzed metathesis reaction has been successfully utilized for the synthesis of carbocyclic, cyclic ethers and cyclic esters. An intermolecular metathesis reaction in solid phase was observed in some instances at high loading levels of the resin, but intramolecular cyclizations are favored at lower loading levels. The methods developed for somatostatin peptides are applicable to other peptides as exemplified by the preparation of neurotensin and bombesin peptides. The reaction conditions are designed to prepare a wide variety of cyclic compounds for functionalization either at the N-terminus or in the macrocyclic ring. Macrocyclic peptides are ideal candidates for Tc-99m chelation chemistry because of the absence of reducible groups, such as disulfide. Methods developed here are amenable to the preparation of a large number of peptides and peptidomimetics by combinatorial chemistry.

I. Endocyclic amines containing a chelating moiety



AA, $AA_2$, $AA_3$ = natural and unnatural amino acids; this includes α-, β- and γ-aminoacids and L- and D- aminoacids;

a, b = 0-10;

k, l = 0-5;

m = 0-20;

n , n' = 1-10;

P is none, O, S, COO, NH-CO, NR, N-CH(=NH)-$NH_2$, NH-CO-NH, NH-COO;

R is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups bearing –OH at any location;

p, p', p'' = 0-10;

Q is none, O, S, COO, NH-CO, NR, N-CH(=NH)-$NH_2$, NH-CO-NH, NH-COO;

E is a group of formula $COOR_4$, $CH_2OR_5$, $CON(R_6)OH$ or $CON(R_7)(R_8)$ wherein

$R_4$ is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups,

$R_5$ is hydrogen or physiologically acceptable, physiologically hydrolyzable ester,

$R_6$ is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups,

$R_7$, $R_8$ is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups or taken together form a cyclic alkyl group $C_3$-$C_{10}$;

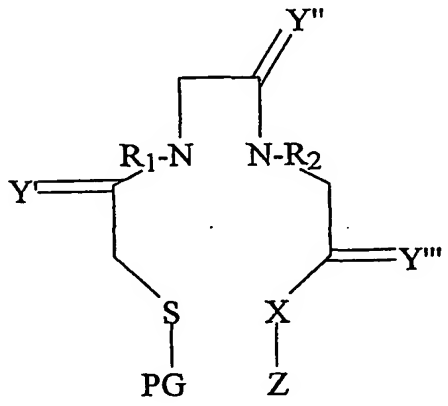
CM is a dye, a therapeutic agent, or a chelating moiety or metal binding site wherein the chelating moiety is labeled with a metal isotope selected from $^{99m}Tc$, $^{203}Pb$, $^{67}Ga$, $^{111}In$, $^{97}Ru$, $^{62}Cu$, $^{64}Cu$, $^{186}Re$, $^{188}Re$, $^{90}Y$, $^{121}Sn$, $^{161}Tb$, $^{153}Sm$, $^{166}Ho$, $^{105}Rh$, $^{177}Lu$ or a radioactive halogen isotope on the understanding that

i) if the label is a metal isotope, CM represents a chelating group suitable for the metal and

ii) if the label is a radioactive halogen isotope, the halogen is attached to an aromatic ring,

wherein the CM is attached directly or through a spacing group to the peptide, said CM being attached to the amine through an amide or urea bond or by any other modification which allows attachment of a chelate and which modifications are known to those of skill in the art,

wherein the chelating group is preferably derived from ethylene diamine tetraacetic acid (EDTA), diethylene triamine pentaacetic acid (DTPA), cyclohexyl 1,2-diamine tetraacetic acid (CDTA), ethyleneglycol-O,O'-bis(2-aminoethyl)-N,N,N',N'-diacetic acid (HBED), triethylene tetraamine hexaacetic acid (TTHA), 1,4,7,10-tetraazacyclododecane-N,N',N",N'"-tetraacetic acid (DOTA), 1,4,7-triazacyclononane-N,N',N"-triacetic acid (NOTA), 1,4,8,11-tetraazacyclotetradecane-N,N',N",N'"-tetraacetic acid (TETA) or a compound with a general formula




wherein

PG is a sulfur protecting group selected from alkanoyl, arylcarbonyl, arylalkanoyl, acetamidomethyl, tetrahydropyranyl and tetrahydrofuranyl,

Y', Y", and Y'" are hydrogen or oxygen with the proviso that at least one of them is an O,

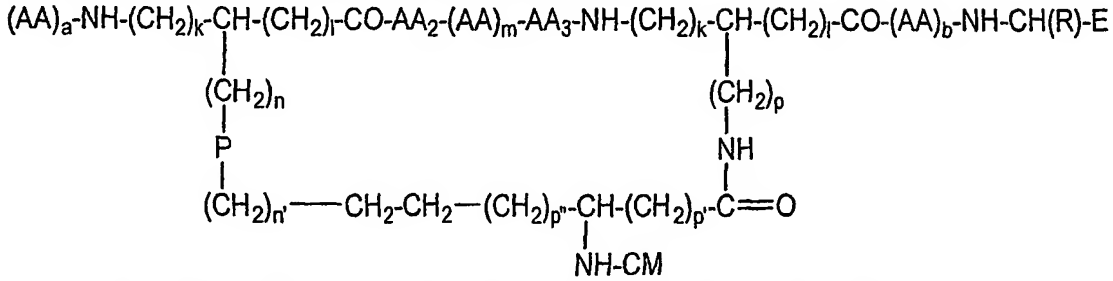
$R_1$ and $R_2$ are hydrogen or alkyl ($C_1$-$C_3$),

X = NH or S with the proviso that Y'" is hydrogen when X is S,

Z is PG if X is S, and

Z is hydroxyalkyl, aminoalkyl or carboxyalkyl.

II. Exocyclic amines containing a chelating moiety



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a, b = 0-10;

k, l = 0-5;

m = 0-20;

n , n' = 1-10;

P none, O, S, COO, NH-CO, NR, N-CH(=NH)-$NH_2$, NH-CO-NH, NH-COO;

R is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups bearing –OH at any location;

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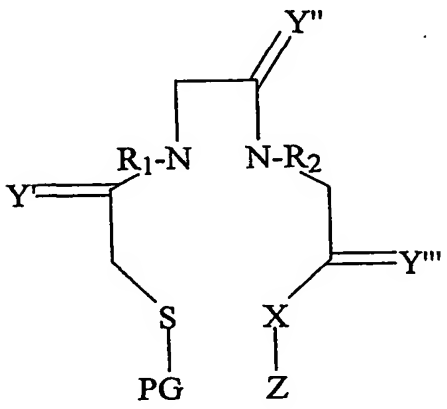


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Y', Y", and Y"' are hydrogen or oxygen with the proviso that at least one of them is an O,

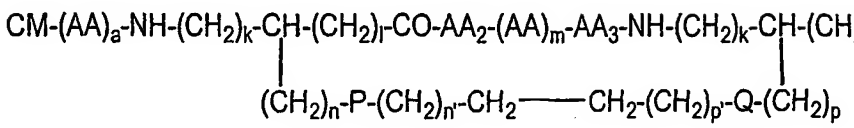
$R_1$ and $R_2$ are hydrogen or alkyl ($C_1$-$C_3$),

X = NH or S with the proviso that Y"' is hydrogen when X is S,

Z is PG if X is S, and

Z is hydroxyalkyl, aminoalkyl or carboxyalkyl.

III. Chelating moiety at the N-terminus



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a, b = 0-10;

k, l = 0-5;

m = 0-20;

n , n' = 1-10;

P, Q is none, O, S, COO, NH-CO, NR, N-CH(=NH)- $NH_2$, NH-CO-NH, NH-COO;

R is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups bearing –OH at any location;

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E is a group of formula $COOR_4$, $CH_2OR_5$, $CON(R_6)OH$ or $CON(R_7)(R_8)$ wherein

$R_4$ is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups,

$R_5$ is hydrogen or physiologically acceptable, physiologically hydrolyzable ester,

$R_6$ is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups,

$R_7$, $R_8$ is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups or taken together form a cyclic alkyl group $C_3$-$C_{10}$;

CM is a dye, a therapeutic agent, or a chelating moiety or metal binding site wherein the chelating moiety is labeled with a metal isotope selected from $^{99m}Tc$, $^{203}Pb$, $^{67}Ga$, $^{111}In$, $^{97}Ru$, $^{62}Cu$, $^{64}Cu$, $^{186}Re$, $^{188}Re$, $^{90}Y$, $^{121}Sn$, $^{161}Tb$, $^{153}Sm$, $^{166}Ho$, $^{105}Rh$, $^{177}Lu$ or a radioactive halogen isotope on the understanding that

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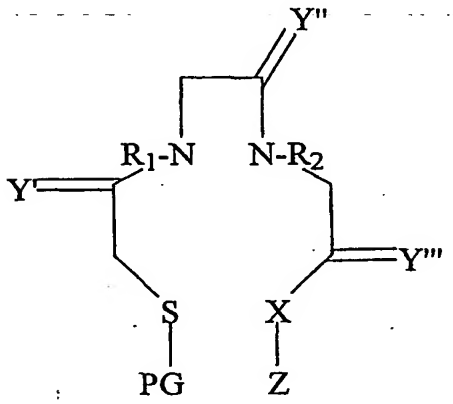
wherein the chelating group is preferably derived from ethylene diamine tetraacetic acid (EDTA), diethylene triamine pentaacetic acid (DTPA), cyclohexyl 1,2-diamine tetraacetic acid (CDTA), ethyleneglycol-O,O'-bis(2-aminoethyl)-N,N,N',N'-diacetic acid (HBED), triethylene tetraamine hexaacetic acid (TTHA), 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA), 1,4,7-triazacyclononane-N,N',N''-triacetic acid (NOTA), 1,4,8,11-tetraazacyclotetradecane-N,N',N'',N'''-tetraacetic acid (TETA) or a compound with a general formula




wherein

PG is a sulfur protecting group selected from alkanoyl, arylcarbonyl, arylalkanoyl, acetamidomethyl, tetrahydropyranyl and tetrahydrofuranyl,

Y', Y'', and Y''' are hydrogen or oxygen with the proviso that at least one of them is an O,

$R_1$ and $R_2$ are hydrogen or alkyl ($C_1$-$C_3$),

X = NH or S with the proviso that Y''' is hydrogen when X is S,

Z is PG if X is S, and

Z is hydroxyalkyl, aminoalkyl or carboxyalkyl.

Throughout this disclosure, the dyes and therapeutics which can be used for CM include, but are not limited to, the following:

Visible dyes:

Fluorescein

Fluorescein isothiocyanate (FITC)

Naphthofluorescein

Rhodamine derivatives

Texas Red

Hydroxycoumarin

Infrared dyes:

Indocyanine Green (ICG)

Bis-propanoic acid cyanine

Photodynamic therapy dyes/photosensitizers:

Acridines (acridine orange, acridine yellow, proflavin, etc.)

Thiazines (methylene blue, azure C, toluidine blue)

Xanthenes (fluorescein, rose Bengal)

Phenazines (neutral red)

Porphyrins

Naphthalimide

Cancer Drugs:

Tamoxifen

Adriamycin

Phillotoxins

Taxol and analogs

Bleomycin

Doxorubicin

Etoposide

Methotrexate

Vinblastine and analogs

Dicarbazine

Actinomycin D

The invention will now be described in greater detail with reference to the following specific Examples, which are offered by way of illustration and are not intended to limit the invention in any manner. Standard techniques well known in the art or the techniques specifically described below are utilized.

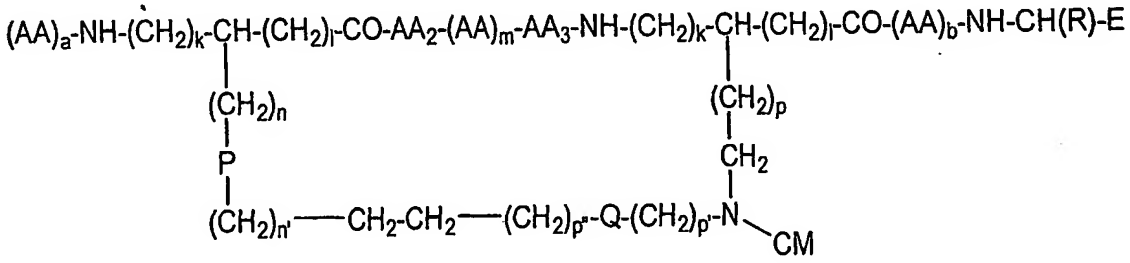
Example 1

Peptide Synthesis

All the linear peptides in the study were prepared by solid phase peptide synthesis employing a Fmoc[9-fluorenylmethoxycarbonyl] strategy. All the amino acids were purchased commercially.

In all the following examples, in all the resin bound peptides, the side chains of the individual amino acids have protecting groups unless otherwise stated.

Example 2



Somatostatins:

$(AA)_a$ is Phe, Tyr, an isomer of Tyr, polyhydroxylated Phe, or aromatic amino acids, wherein the amino acid can have an L- or D- configuration;

k is 1, 2 or 3;

l is 1, 2 or 3;

$AA_2$ is Phe, Tyr, an isomer of Tyr, polyhydroxylated Phe, or aromatic amino acids, wherein the amino acid can have an L- or D- configuration;

$(AA)_m$ is a dipeptide sequence consisting of DTrp-Lys, DTrp-Orn, DTrp-Dab, DTrp-4-piperidinylglycine, DTrp-4-piperidinylalanine, DTrp-4-aminomethylcyclohexylalanine, DTrp-4-aminomethylcyclohexylglycine, DTrp-4-aminocyclohexylalanine, DTrp-4-aminocyclohexylglycine. DTrp can be substituted by L-Trp;

$AA_3$ is any amino acid;

$(AA)_b$ is none, serine or threonine;

R is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups bearing –OH at any location;

E is COOH, $CH_2$-OH, $CONH_2$, $COOR_4$ or CONHOH wherein $R_4$ is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups;

n is 1, 2 or 3;

P is none, O or S;

n' is 1-7;

p is 1-6;

p' is 1-6;

p'' is 1-6;

Q is none, O or S;

CM is a dye, a therapeutic agent, or a chelating moiety or metal binding site wherein the chelating moiety is labeled with a metal isotope selected from $^{99m}Tc$, $^{203}Pb$, $^{67}Ga$, $^{111}In$, $^{97}Ru$, $^{62}Cu$, $^{64}Cu$, $^{186}Re$, $^{188}Re$, $^{90}Y$, $^{121}Sn$, $^{161}Tb$, $^{153}Sm$, $^{166}Ho$, $^{105}Rh$, $^{177}Lu$ or a radioactive halogen isotope on the understanding that

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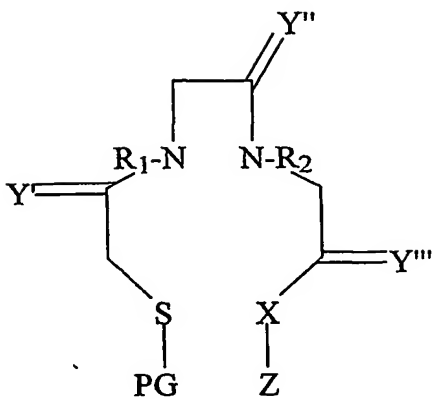
wherein the CM is attached directly or through a spacing group to the peptide, said CM being attached to the amine through an amide or urea bond or by any other modification which allows attachment of a chelate and which modifications are known to those of skill in the art,

wherein the chelating group is preferably derived from ethylene diamine tetraacetic acid (EDTA), diethylene triamine pentaacetic acid (DTPA), cyclohexyl 1,2-diamine tetraacetic acid (CDTA), ethyleneglycol-O,O'-bis(2-aminoethyl)-N,N,N',N'-diacetic acid (HBED), triethylene tetraamine hexaacetic acid (TTHA), 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA), 1,4,7-triazacyclononane-N,N',N''-triacetic acid (NOTA), 1,4,8,11-tetraazacyclotetradecane-N,N',N'',N'''-tetraacetic acid (TETA) or a compound with a general formula

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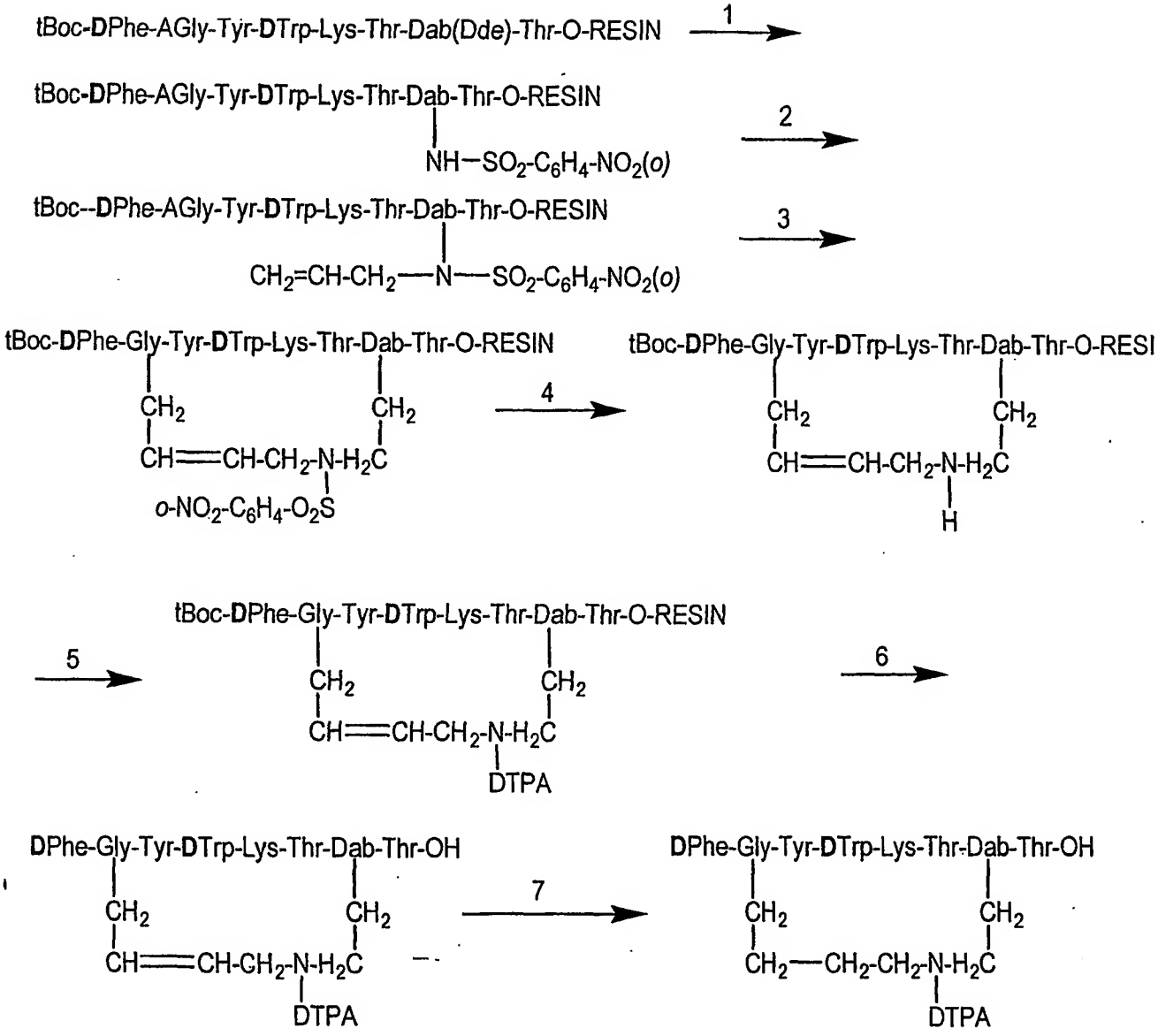

tBoc-DPhe-AGly-Tyr-DTrp-Lys-Thr-Dab(Dde)-Thr-O-RESIN
1
tBoc-DPhe-AGly-Tyr-DTrp-Lys-Thr-Dab-Thr-O-RESIN
NH—SO2-C6H4-NO2(o)
2
tBoc--DPhe-AGly-Tyr-DTrp-Lys-Thr-Dab-Thr-O-RESIN
CH2=CH-CH2—N—SO2-C6H4-NO2(o)
3
tBoc-DPhe-Gly-Tyr-DTrp-Lys-Thr-Dab-Thr-O-RESIN
CH2
CH2
CH=CH-CH2-N-H2C
o-NO2-C6H4-O2S
4
tBoc-DPhe-Gly-Tyr-DTrp-Lys-Thr-Dab-Thr-O-RESI
CH2
CH2
CH=CH-CH2-N-H2C
H
5
tBoc-DPhe-Gly-Tyr-DTrp-Lys-Thr-Dab-Thr-O-RESIN
CH2
CH2
CH=CH-CH2-N-H2C
DTPA
6
DPhe-Gly-Tyr-DTrp-Lys-Thr-Dab-Thr-OH
CH2
CH2
CH=CH-CH2-N-H2C
DTPA
7
DPhe-Gly-Tyr-DTrp-Lys-Thr-Dab-Thr-OH
CH2
CH2
CH2—CH2-CH2-N-H2C
DTPA

Step 1: The protected peptide was assembled in an automated synthesizer according to the Fmoc-strategy. The resin (Wang) bound peptide was shaken with 2% hydrazine (2 mL hydrazine per 50 mg of resin) for 30 minutes to remove the Dde protecting group, followed by protection of the side chain amino group with o-nitrobenzenesulfonyl group (NBS) using commercially available o-nitrobenzenesulfonyl chloride in the presence diisopropylethylamine (DIEA).

Step 2: The resin, tBoc-DPhe$^1$,AGly$^2$,Tyr$^3$,Dab$^7$(β-o-NBS)-Octreotate-Resin (55 mg, 10 μmol peptide content, 0.18 mmol/g) was suspended in a solution of 1 mL of methylene chloride $CH_2Cl_2$ containing 52 mg of triphenyl phosphine ($Ph_3P$) (0.2 mmol; 20 Xs.) 34 μL of diethylazodicarboxylate (DEAD) (0.2 mmol; 20 Xs.). After vigorous shaking for a few minutes, allyl alcohol (20 fold excess) was added. After vortexing for overnight, the resin was filtered, washed with 5 mL of methylene chloride and dried. In similar reactions, the resin-bound peptide was alkylated with 3-butenol, 4-pentenol, 5-hexenol or allyloxyethanol. In each case, a small amount of the peptide was cleaved from the resin and assayed to ensure complete alkylation.

Step3: 50 mg of the resin (25 μmole peptide) was suspended in 5 mL of methylene chloride containing 20 mg of Grubbs' catalyst. The mixture was heated at 40°C for 10-15 hours. At the end of the reaction, the resin was removed by filtration and washed with methylene chloride and THF (tetrahydrofuran).

Step 4: The resin (250 mg; 0.18 mmol/g) containing the previously made peptide of step 3 was suspended in 3 mL of DMF (dimethylformamide). To this suspension, 200 μL of DBU and 200 μL of mercaptoethanol was added and shaken for 7 hours.

Step 5: A solution of tri-t-butyl-DTPA anhydride (56 mg; 0.1 mmol) in 200 μL of DMF was activated with 0.5 mL of HOBt-HBTU (200 mM) solution for 1 hour and added to 140 mg (50 μmol of the peptide) of the above resin. The suspension was shaken for overnight and filtered. The resin was washed with DMF and 10 mL of THF.

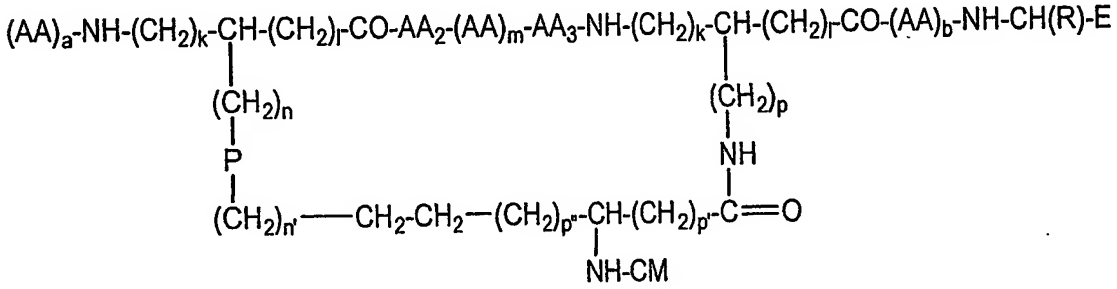
Step 6: The resin was deprotected using 250 μL of TFA:phenol:thioanisole:water (85:5:5:5) overnight. The crude peptide was precipitated using 10 mL of MeOtBu. After centrifugation, the mixture was washed with 4 X 10 mL of dissolved in MeOtBu. The mixture was taken up in 2 mL of 2:3 acetonitrile:water, shaken in a vortex mixer and the resin was removed by filtration. The filtrate was lyophilized to obtain the peptide.

Step 7: The compound (~6 mg) was dissolved in 8 mL of MeOH:$H_2O$ (0.001 M HCl) (1:1). The solution was hydrogenated in the presence of 1-2 mg of $PtO_2$ (Adams' catalyst) for 10-12 hours. Catalyst was filtered and the solution was evaporated to dryness. The residue was dissolved in 1-2 mL of water and evaporated and the process was repeated two more times. The residue was dissolved in water and lyophilized to obtained the product.

Example 3

Somatostatins

$(AA)_a$ is Phe, Tyr, an isomer of Tyr, polyhydroxylated Phe or aromatic amino acids, wherein the amino acid can have an L- or D- configuration;



k is 1, 2 or 3;

l is 1, 2 or 3;

$AA_2$ is Phe, Tyr, an isomer of Tyr, polyhydroxylated Phe or aromatic amino acids, wherein the amino acid can have an L- or D- configuration;

$(AA)_m$ is a dipeptide sequence consisting of DTrp-Lys, DTrp-Orn, DTrp-Dab, DTrp-4-piperidinylglycine, DTrp-4-piperidinylalanine, DTrp-4-aminomethylcyclohexy-lalanine, DTrp-4-aminomethylcyclohexylglycine, DTrp-4-aminocyclohexylalanine, DTrp-4-aminocyclohexylglycine and DTrp can be substituted by L-Trp;

$AA_3$ is any amino acid;

$(AA)_b$ is none, serine or threonine;

R is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups bearing –OH at any location;

E is COOH, $CH_2$-OH, $CONH_2$, $COOR_4$ or CONHOH wherein $R_4$ is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups;

n is 1, 2 or 3;

P is none, O or S;

n' is 1-7;

p is 1-6;

p' is 1-6;

p'' is 1-6;

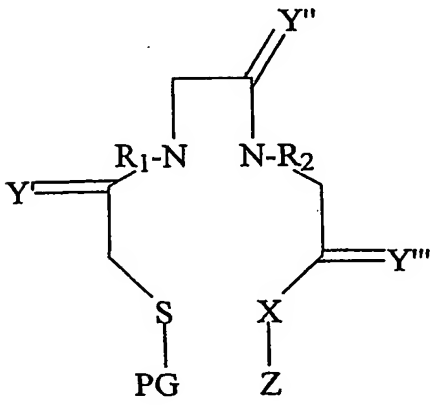
CM is a dye, a therapeutic agent, or a chelating moiety or metal binding site wherein the chelating moiety is labeled with a metal isotope selected from $^{99m}Tc$, $^{203}Pb$, $^{67}Ga$, $^{111}In$, $^{97}Ru$, $^{62}Cu$, $^{64}Cu$, $^{186}Re$, $^{188}Re$, $^{90}Y$, $^{121}Sn$, $^{161}Tb$, $^{153}Sm$, $^{166}Ho$, $^{105}Rh$, $^{177}Lu$ or a radioactive halogen isotope on the understanding that

i) if the label is a metal isotope, CM represents a chelating group suitable for the metal and

ii) if the label is a radioactive halogen isotope, the halogen is attached to an aromatic ring,

wherein the CM is attached directly or through a spacing group to the peptide, said CM being attached to the amine through an amide or urea bond or by any other modification which allows attachment of a chelate and which modifications are known to those of skill in the art,

wherein the chelating group is preferably derived from ethylene diamine tetraacetic acid (EDTA), diethylene triamine pentaacetic acid (DTPA), cyclohexyl 1,2-diamine tetraacetic acid (CDTA), ethyleneglycol-O,O'-bis(2-aminoethyl)-N,N,N',N'-diacetic acid (HBED), triethylene tetraamine hexaacetic acid (TTHA), 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA), 1,4,7-triazacyclononane-N,N',N''-triacetic acid (NOTA), 1,4,8,11-tetraazacyclotetradecane-N,N',N'',N'''-tetraacetic acid (TETA) or a compound with a general formula



wherein

PG is a sulfur protecting group selected from alkanoyl, arylcarbonyl, arylalkanoyl, acetamidomethyl, tetrahydropyranyl and tetrahydrofuranyl,

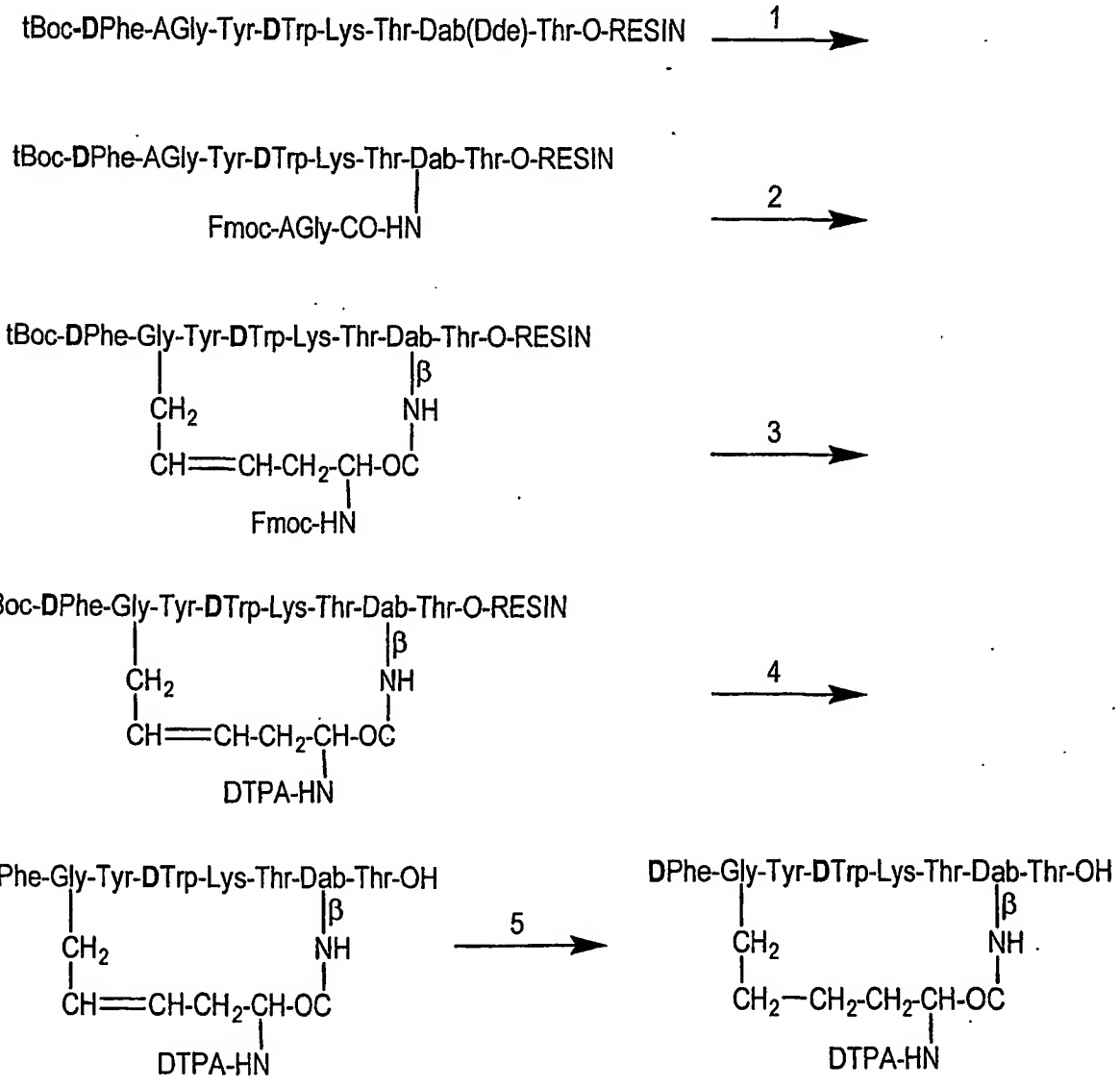
Y', Y", and Y"' are hydrogen or oxygen with the proviso that at least one of them is an O,

$R_1$ and $R_2$ are hydrogen or alkyl ($C_1$-$C_3$),

X = NH or S with the proviso that Y"' is hydrogen when X is S,

Z is PG if X is S, and

Z is hydroxyalkyl, aminoalkyl or carboxyalkyl.



Step 1: The protected peptide was assembled in an automated synthesizer according to the Fmoc-strategy. The resin bound peptide was shaken with 2% hydrazine (2 mL per 50 mg resin) for 30 minutes to remove the Dde protecting group, followed by reaction with Fmoc-L-allylglycine activated ester (4 fold excess) to give the product.

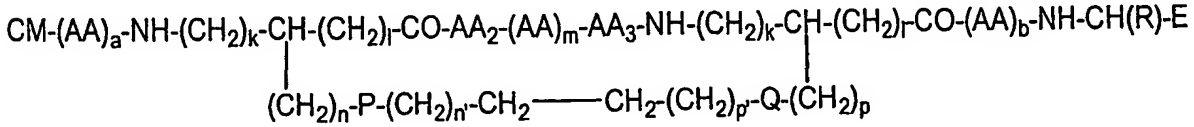
Step 2: 50 mg of the resin (25 μmole peptide) was suspended in 5 mL of methylene chloride containing 20 mg of Grubbs' catalyst. The mixture was heated at 40°C for 10-15 hours. At the end of the reaction, the resin was removed by filtration and washed with methylene chloride and THF.

Step 3: The resin was shaken with 1:1 piperidine:DMF (1 mL per 50 mg resin) for 1 hour. After the resin was filtered it was washed with THF and dried. A solution of tri-t-butyl-DTPA anhydride (56 mg; 0.1 mmol) in 200 μL of DMF was activated with 0.5 mL of HOBt-HBTU (200 mM) solution for 1 hour and added to 140 mg (50 μmol of the peptide) of the above resin. The suspension was shaken for overnight and filtered. The resin was washed with DMF and 10 mL of THF.

Step 4: The resin was deprotected using 250 μL of TFA:phenol:thioanisole:water (85:5:5:5) overnight. The crude peptide was precipitated using 10 mL of MeOtBu. After centrifugation, the mixture was washed with 4 X 10 mL of MeOtBu. The mixture was taken up in 2 mL of 2:3 acetonitrile:water, shaken in a vortex mixer and the resin was removed by filtration. The filtrate was lyophilized to obtain the peptide.

Step 5: The compound (~5 mg) was dissolved in 10 mL of MeOH:$H_2O$ (0.001M HCl) (1:1). The solution was hydrogenated in the presence of 1-2 mg of $PtO_2$ (Adams' catalyst) for 10-12 hours. Catalyst was filtered and the solution was evaporated to dryness. The residue was dissolved in 1-2 mL of water and evaporated and the process was repeated two more times. The residue was dissolved in water and lyophilized to obtained the product.

Example 4




AA, $AA_2$, $AA_3$ = natural and unnatural amino acids; this includes α-, β- and γ-aminoacids and L- and D- aminoacids;

a, b = 0-10;

k, l = 0-5;

m = 0-20;

n , n'. = 1-10;

P, Q is none, O, S, COO, NH-CO, NR, N-CH(=NH)- $NH_2$, NH-CO-NH, NH-COO;

R is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups bearing –OH at any location;

p, p' = 0-10;

E is a group of formula $COOR_4$, $CH_2OR_5$, $CON(R_6)OH$, $CON(R_7)(R_8)$ wherein

$R_4$ is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups,

$R_5$ is hydrogen or physiologically acceptable, physiologically hydrolyzable ester,

$R_6$ is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups,

$R_7$, $R_8$ is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups or taken together form a cyclic alkyl group $C_3$-$C_{10}$;

CM is a dye, a therapeutic agent, or a chelating moiety or metal binding site wherein the chelating moiety is labeled with a metal isotope selected from $^{99m}Tc$, $^{203}Pb$, $^{67}Ga$, $^{111}In$, $^{97}Ru$, $^{62}Cu$, $^{64}Cu$, $^{186}Re$, $^{188}Re$, $^{90}Y$, $^{121}Sn$, $^{161}Tb$, $^{153}Sm$, $^{166}Ho$, $^{105}Rh$, $^{177}Lu$ or a radioactive halogen isotope on the understanding that

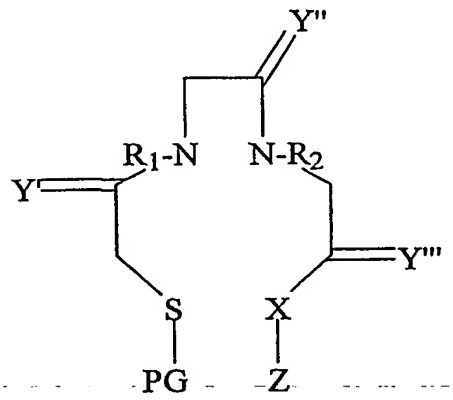
i) if the label is a metal isotope, CM represents a chelating group suitable for the metal and

ii) if the label is a radioactive halogen isotope, the halogen is attached to an aromatic ring,

wherein the CM is attached directly or through a spacing group to the peptide, said CM being attached to the amine through an amide or urea bond or by any other modification which allows attachment of a chelate and which modifications are known to those of skill in the art,

wherein the chelating group is preferably derived from ethylene diamine tetraacetic acid (EDTA), diethylene triamine pentaacetic acid (DTPA), cyclohexyl 1,2-diamine tetraacetic acid (CDTA), ethyleneglycol-O,O'-bis(2-aminoethyl)-N,N,N',N'-diacetic acid (HBED), triethylene tetraamine hexaacetic acid (TTHA), 1,4,7,10-tetraazacyclododecane-N,N',N",N"'-tetraacetic acid (DOTA), 1,4,7-triazacyclononane-N,N',N"-triacetic acid

(NOTA), 1,4,8,11-tetraazacyclotetradecane-N,N',N'',N'''-tetraacetic acid (TETA) or a compound with a general formula




wherein

PG is a sulfur protecting group selected from alkanoyl, arylcarbonyl, arylalkanoyl, acetamidomethyl, tetrahydropyranyl and tetrahydrofuranyl,

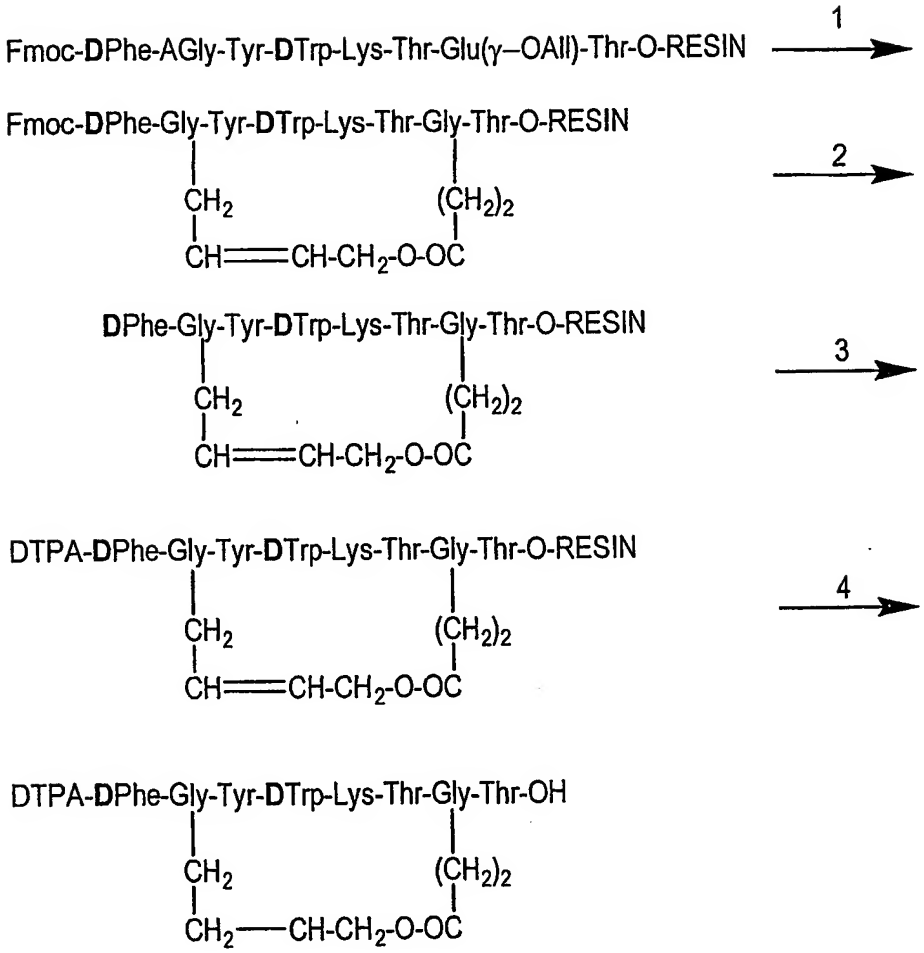
Y', Y'', and Y''' are hydrogen or oxygen with the proviso that at least one of them is an O,

$R_1$ and $R_2$ are hydrogen or alkyl ($C_1$-$C_3$),

X = NH or S with the proviso that Y''' is hydrogen when X is S,

Z is PG if X is S, and

Z is hydroxyalkyl, aminoalkyl or carboxyalkyl.



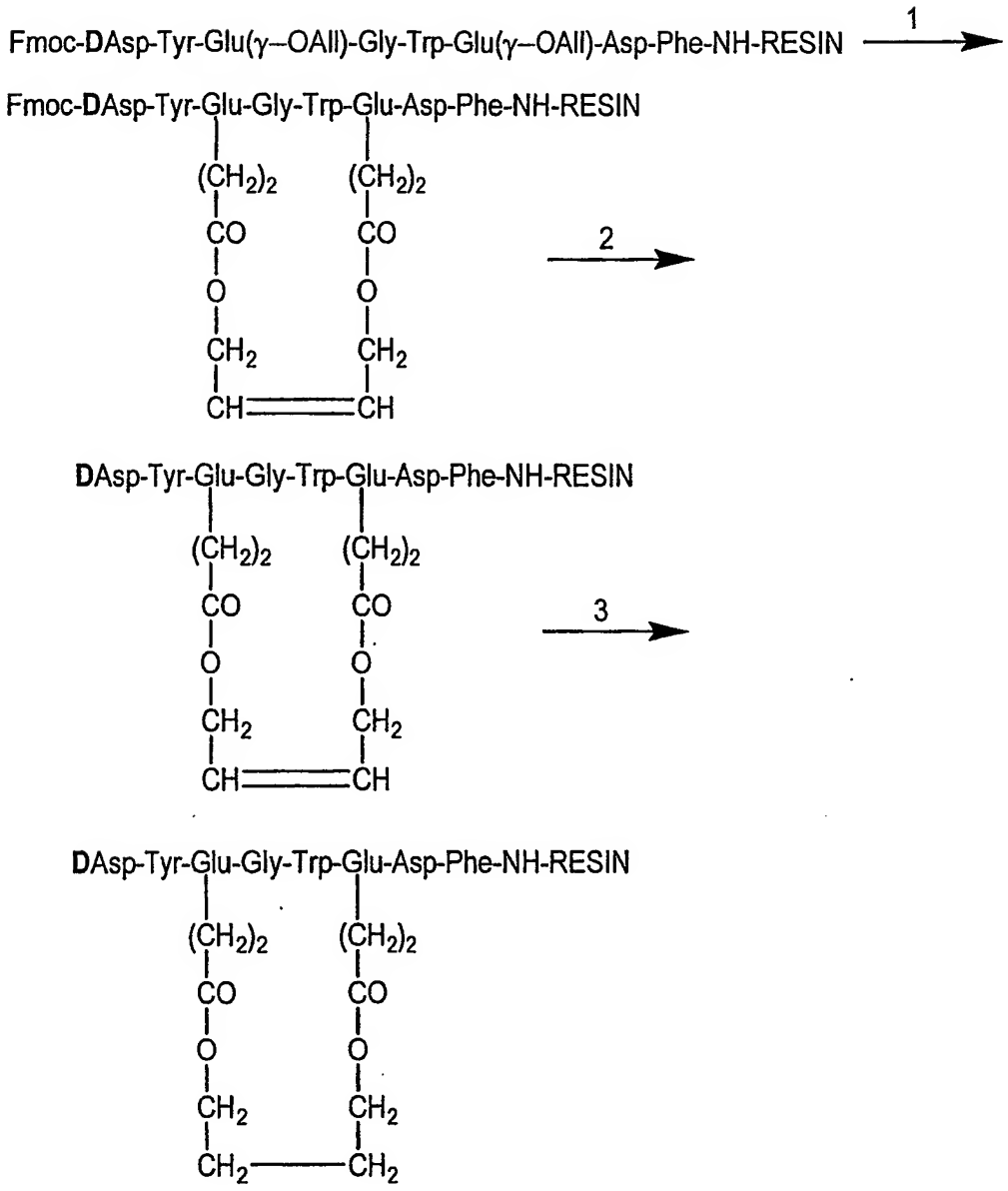

Step 1: 500 mg of the resin (90 μmole peptide) was suspended in 22 mL of methylene chloride containing 90 mg of Grubbs' catalyst. The mixture was heated at 40°C for 10 hours. At the end of the reaction, the resin was removed by filtration and washed with methylene chloride and THF.

Step 2: The resin containing the cyclic product was treated with 5 mL of 1:1 piperidine:DMF for 30 minutes and filtered. The resin was washed with DMF and 10 mL of anhydrous THF and dried.

Step 3: A solution of tri-t-butyl-DTPA anhydride (112 mg; 0.2 mmol) in 200 μL of DMF was activated with 1 mL of HOBt-HBTU (200 mM) solution for 1 hour and added to 277 mg (50 μmol of the peptide) of the above resin. The suspension was shaken for overnight and filtered. The resin was washed with DMF and 10 mL of THF.

Step 4: The resin (9 μmole; 50 mg; 0.18 mmol/g) was suspended in a solution of 1 mL of DMF containing 30 mg (180 μmole) of p-fluorobenzenesulfonylhydrazide and heated at 75°C for 6 hours. The resin was filtered, washed successively with 5 mL each of DMF and THF and dried. The deprotections were accomplished by using 250 μL of TFA:phenol:thioanisole:water (85:5:5:5) overnight. The crude peptide was precipitated using 10 mL of MeOtBu. After centrifugation, the mixture was washed with 4 X 10 mL of MeOtBu. The mixture was taken up in 2 mL of 2:3 acetonitrile:water, shaken in a vortex mixer and the resin was removed by filtration. The filtrate was lyophilized to obtain the peptide.

In a similar fashion, the following reactions were performed illustrating the use of these reactions to form a macrocycle containing two ester bonds (i.e., both P and Q are esters). Only some of the reaction steps are shown and are described below. Addition of a dye, therapeutic agent or chelating moiety can be performed as described above. This illustrates the generality of the reactions.




Step 1: 500 mg of the resin (90 μmole peptide) was suspended in 22 mL of methylene chloride containing 90 mg of Grubbs' catalyst. The mixture was heated at 40°C for 10 hours. At the end of the reaction, the resin was removed by filtration and washed with methylene chloride and THF.

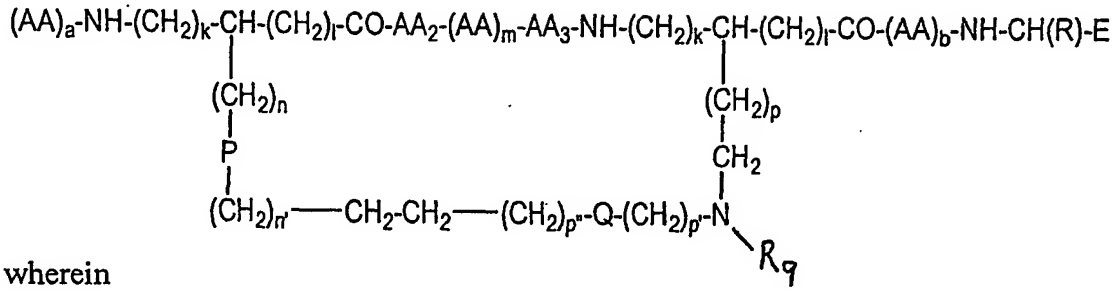
Step 2: The resin containing the cyclic product was treated with 5 mL of 1:1 piperidine:DMF for 30 minutes and filtered. The resin was washed with DMF and 10 mL of anhydrous THF and dried.

Step 3: The resin (9 μmole; 50 mg; 0.18 mmol/g) was suspended in a solution of 1 mL of DMF containing 30 mg (180 μmole) of p-fluorobenzenesulfonylhydrazide and heated at 75°C for 6 hours. The resin was filtered, washed successively with 5 mL each of DMF and THF and dried. The deprotections were accomplished by using 250 μL of TFA:phenol:thioanisole:water (85:5:5:5) overnight. The crude peptide was precipitated using 10 mL of MeOtBu. After centrifugation, the mixture was washed with 4 X 10 mL of MeOtBu. The mixture was taken up in 2 mL of 2:3 acetonitrile:water, shaken in a vortex mixer and the resin was removed by filtration. The filtrate was lyophilized to obtain the peptide.

While the invention has been disclosed in this patent application by reference to the details of preferred embodiments of the invention, it is to be understood that the disclosure is intended in an illustrative rather than in a limiting sense, as it is contemplated that modifications will readily occur to those skilled in the art, within the spirit of the invention and the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A peptide of formula



wherein

AA, $AA_2$, $AA_3$ are natural or unnatural amino acids comprising α-, β- and γ-aminoacids and L- and D- aminoacids;

a, b = 0-10;

k, l = 0-5;

m = 0-20;

n , n' = 1-10;

P is none, O, S, COO, NH-CO, NR, N-CH(=NH)-$NH_2$, NH-CO-NH, NH-COO;

R is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups bearing –OH at any location;

p, p', p'' = 0-10;

Q is none, O, S, COO, NH-CO, NR, N-CH(=NH)-$NH_2$;

E is a group of formula $COOR_4$, $CH_2OR_5$, $CON(R_6)OH$ or $CON(R_7)(R_8)$ wherein

$R_4$ is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups,

$R_5$ is hydrogen or physiologically acceptable, physiologically hydrolyzable ester,

$R_6$ is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups,

$R_7$, $R_8$ is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups or taken together form a cyclic alkyl group $C_3$-$C_{10}$; and

$R_9$ is H, a dye, a therapeutic agent, a chelating moiety or a metal binding site.

2. The peptide of claim 1 wherein said chelating moiety or metal binding site is CM and CM is labeled with a metal isotope selected from $^{99m}Tc$, $^{203}Pb$, $^{67}Ga$, $^{111}In$, $^{97}Ru$, $^{62}Cu$,

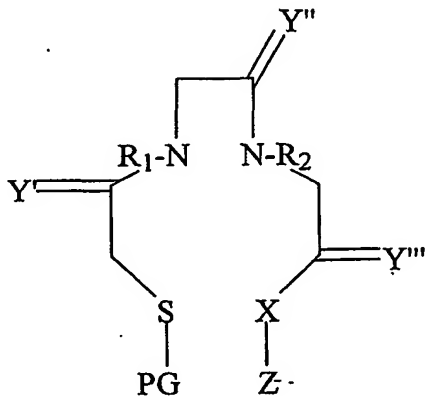
$^{64}Cu$, $^{186}Re$, $^{188}Re$, $^{90}Y$, $^{121}Sn$, $^{161}Tb$, $^{153}Sm$, $^{166}Ho$, $^{105}Rh$, $^{177}Lu$ or a radioactive halogen isotope on the understanding that

i) if the label is a metal isotope, CM represents a chelating group suitable for the metal and

ii) if the label is a radioactive halogen isotope, the halogen is attached to an aromatic ring,

wherein the CM is attached directly or through a spacing group to the peptide, said CM being attached to the amine through an amide or urea bond or by any other modification which allows attachment of a chelate and which modifications are known to those of skill in the art,

wherein the chelating group is preferably derived from ethylene diamine tetraacetic acid (EDTA), diethylene triamine pentaacetic acid (DTPA), cyclohexyl 1,2-diamine tetraacetic acid (CDTA), ethyleneglycol-O,O'-bis(2-aminoethyl)-N,N,N',N'-diacetic acid (HBED), triethylene tetraamine hexaacetic acid (TTHA), 1,4,7,10-tetraazacyclododecane-N,N',N",N"'-tetraacetic acid (DOTA), 1,4,7-triazacyclononane-N,N',N"-triacetic acid (NOTA), 1,4,8,11-tetraazacyclotetradecane-N,N',N",N"'-tetraacetic acid (TETA) or a compound with a general formula




wherein

PG is a sulfur protecting group selected from alkanoyl, arylcarbonyl, arylalkanoyl, acetamidomethyl, tetrahydropyranyl and tetrahydrofuranyl,

Y', Y", and Y"' are hydrogen or oxygen with the proviso that at least one of them is an O,

$R_1$ and $R_2$ are hydrogen or alkyl ($C_1$-$C_3$),

X = NH or S with the proviso that Y''' is hydrogen when X is S,

Z is PG if X is S, and

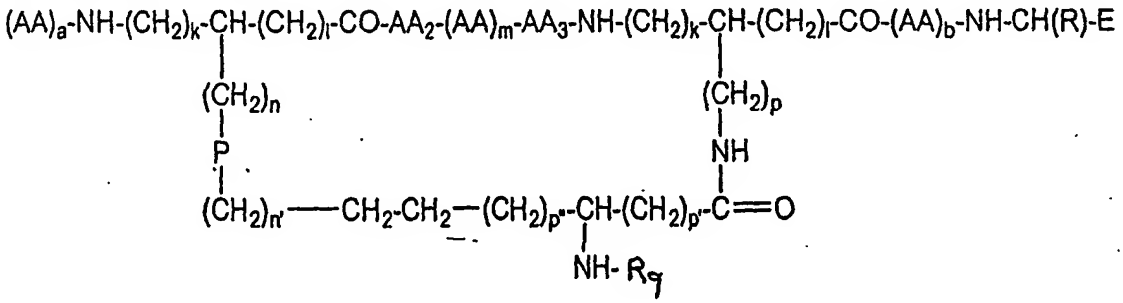
Z is hydroxyalkyl, aminoalkyl or carboxyalkyl.

3. The peptide of claim 1 wherein said dye is selected from the group consisting of fluorescein, fluorescein isothiocyanate, naphthofluorescein, rhodamine derivatives, Texas Red, hydroxycoumarin, indocyanine green, bis-propanoic acid cyanine, acridines, thiazines, phenazines, porphyrins and naphthalimide.

4. The peptide of claim 1 wherein said therapeutic agent is selected from the group consisting of tamoxifen, adriamycin, phillotoxins, taxol, taxol analogs, bleomycin, doxorubicin, etoposide, methotrexate, vinblastine, vinblastine analogs, dicarbazine and actinomycin D.

5. The peptide of claim 1 wherein said peptide is a derivative of: somatostatin, gastrin, gastrin releasing peptide, bombesin, a bombesin antagonist, a gastrin releasing peptide, an adhesion peptide, cholecystokinin, a neurotensin, neuropeptide Y, a vasoactive intestinal peptide, thyroid stimulating hormone, angiotensin, pancreatic adenylate cyclase activating peptide or substance P.

6. A peptide of formula



wherein

AA, $AA_2$, $AA_3$ are natural or unnatural amino acids comprising α-, β- or γ-aminoacids, and L- or D- aminoacids;

a, b = 0-10;

k, l = 0-5;

m = 0-20;

n , n' = 1-10;

P is none, O, S, COO, NH-CO, NR, N-CH(=NH)-$NH_2$, NH-CO-NH, NH-COO;

R is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups bearing –OH at any location;

p, p', p'' = 0-10;

E is a group of formula $COOR_4$, $CH_2OR_5$, $CON(R_6)OH$ or $CON(R_7)(R_8)$ wherein

$R_4$ is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups,

$R_5$ is hydrogen or physiologically acceptable, physiologically hydrolyzable ester,

$R_6$ is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups,

$R_7$, $R_8$ is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups or taken together form a cyclic alkyl group $C_3$-$C_{10}$; and

$R_9$ is H, a dye, a therapeutic agent, a chelating moiety or a metal binding site.

7. The peptide of claim 6 wherein said chelating moiety or metal binding site is CM and CM is labeled with a metal isotope selected from $^{99m}Tc$, $^{203}Pb$, $^{67}Ga$, $^{111}In$, $^{97}Ru$, $^{62}Cu$, $^{64}Cu$, $^{186}Re$, $^{188}Re$, $^{90}Y$, $^{121}Sn$, $^{161}Tb$, $^{153}Sm$, $^{166}Ho$, $^{105}Rh$, $^{177}Lu$ or a radioactive halogen isotope on the understanding that

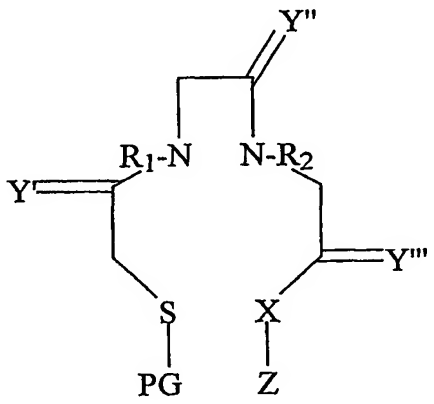
i) if the label is a metal isotope, CM represents a chelating group suitable for the metal and

ii) if the label is a radioactive halogen isotope, the halogen is attached to an aromatic ring,

wherein the CM is attached directly or through a spacing group to the peptide, said CM being attached to the amine through an amide or urea bond or by any other modification which allows attachment of a chelate and which modifications are known to those of skill in the art,

wherein the chelating group is preferably derived from ethylene diamine tetraacetic acid (EDTA), diethylene triamine pentaacetic acid (DTPA), cyclohexyl 1,2-diamine tetraacetic acid (CDTA), ethyleneglycol-O,O'-bis(2-aminoethyl)-N,N,N',N'-diacetic acid (HBED), triethylene tetraamine hexaacetic acid (TTHA), 1,4,7,10-

tetraazacyclododecane-N,N',N",N'"-tetraacetic acid (DOTA), 1,4,7-triazacyclononane-N,N',N"-triacetic acid (NOTA), 1,4,8,11-tetraazacyclotetradecane-N,N',N",N'"-tetraacetic acid (TETA) or a compound with a general formula




wherein

PG is a sulfur protecting group selected from alkanoyl, arylcarbonyl, arylalkanoyl, acetamidomethyl, tetrahydropyranyl and tetrahydrofuranyl,

Y', Y", and Y'" are hydrogen or oxygen with the proviso that at least one of them is an O,

$R_1$ and $R_2$ are hydrogen or alkyl ($C_1$-$C_3$),

X = NH or S with the proviso that Y'" is hydrogen when X is S,

Z is PG if X is S, and

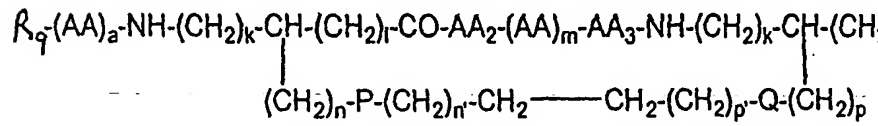
Z is hydroxyalkyl, aminoalkyl or carboxyalkyl.

8. The peptide of claim 6 wherein said dye is selected from the group consisting of fluorescein, fluorescein isothiocyanate, naphthofluorescein, rhodamine derivatives, Texas Red, hydroxycoumarin, indocyanine green, bis-propanoic acid cyanine, acridines, thiazines, phenazines, porphyrins and naphthalimide.

9. The peptide of claim 6 wherein said therapeutic agent is selected from the group consisting of tamoxifen, adriamycin, phillotoxins, taxol, taxol analogs, bleomycin, doxorubicin, etoposide, methotrexate, vinblastine, vinblastine analogs, dicarbazine and actinomycin D.

10. The peptide of claim 6 wherein said peptide is a derivative of: somatostatin, gastrin, gastrin releasing peptide, bombesin, a bombesin antagonist, a gastrin releasing peptide, an adhesion peptide, cholecystokinin, a neurotensin, neuropeptide Y, a vasoactive intestinal peptide, thyroid stimulating hormone, angiotensin, pancreatic adenylate cyclase activating peptide or substance P.

11. A peptide of formula



wherein

AA, $AA_2$, $AA_3$ are natural and unnatural amino acids comprising α-, β- or γ-aminoacids and L- and D- aminoacids;

a, b = 0-10;

k, l = 0-5;

m = 0-20;

n , n' = 1-10;

P, Q is none, O, S, COO, NH-CO, NR, N-CH(=NH)- $NH_2$, NH-CO-NH, NH-COO;

R is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups bearing –OH at any location;

p, p' = 0-10;

E is a group of formula $COOR_4$, $CH_2OR_5$, $CON(R_6)OH$ or $CON(R_7)(R_8)$ wherein

$R_4$ is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups,

$R_5$ is hydrogen or physiologically acceptable, physiologically hydrolyzable ester,

$R_6$ is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups,

$R_7$, $R_8$ is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups or taken together form a cyclic alkyl group $C_3$-$C_{10}$; and

$R_9$ is H, a dye, a therapeutic agent, a chelating moiety or a metal binding site.

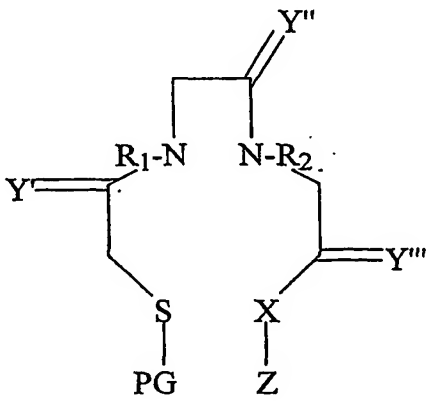
12. The peptide of claim 11 wherein said chelating moiety or metal binding site is CM wherein CM is labeled with a metal isotope selected from $^{99m}Tc$, $^{203}Pb$, $^{67}Ga$, $^{111}In$, $^{97}Ru$, $^{62}Cu$, $^{64}Cu$, $^{186}Re$, $^{188}Re$, $^{90}Y$, $^{121}Sn$, $^{161}Tb$, $^{153}Sm$, $^{166}Ho$, $^{105}Rh$, $^{177}Lu$ or a radioactive halogen isotope on the understanding that

i) if the label is a metal isotope, CM represents a chelating group suitable for the metal and

ii) if the label is a radioactive halogen isotope, the halogen is attached to an aromatic ring,

wherein the CM is attached directly or through a spacing group to the peptide, said CM being attached to the amine through an amide or urea bond or by any other modification which allows attachment of a chelate and which modifications are known to those of skill in the art,

wherein the chelating group is preferably derived from ethylene diamine tetraacetic acid (EDTA), diethylene triamine pentaacetic acid (DTPA), cyclohexyl 1,2-diamine tetraacetic acid (CDTA), ethyleneglycol-O,O'-bis(2-aminoethyl)-N,N,N',N'-diacetic acid (HBED), triethylene tetraamine hexaacetic acid (TTHA), 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA), 1,4,7-triazacyclononane-N,N',N''-triacetic acid (NOTA), 1,4,8,11-tetraazacyclotetradecane-N,N',N'',N'''-tetraacetic acid (TETA) or a compound with a general formula




wherein

PG is a sulfur protecting group selected from alkanoyl, arylcarbonyl, arylalkanoyl, acetamidomethyl, tetrahydropyranyl and tetrahydrofuranyl,

Y', Y", and Y"' are hydrogen or oxygen with the proviso that at least one of them is an O,

$R_1$ and $R_2$ are hydrogen or alkyl ($C_1$-$C_3$),

X = NH or S with the proviso that Y"' is hydrogen when X is S,

Z is PG if X is S, and

Z is hydroxyalkyl, aminoalkyl or carboxyalkyl.

13. The peptide of claim 11 wherein said dye is selected from the group consisting of fluorescein, fluorescein isothiocyanate, naphthofluorescein, rhodamine derivatives, Texas Red, hydroxycoumarin, indocyanine green, bis-propanoic acid cyanine, acridines, thiazines, phenazines, porphyrins and naphthalimide.

14. The peptide of claim 11 wherein said therapeutic agent is selected from the group consisting of tamoxifen, adriamycin, phillotoxins, taxol, taxol analogs, bleomycin, doxorubicin, etoposide, methotrexate, vinblastine, vinblastine analogs, dicarbazine and actinomycin D.

15. The peptide of claim 11 wherein said peptide is a derivative of: somatostatin, gastrin, gastrin releasing peptide, bombesin, a bombesin antagonist, a gastrin releasing peptide, an adhesion peptide, cholecystokinin, a neurotensin, neuropeptide Y, a vasoactive intestinal peptide, thyroid stimulating hormone, angiotensin, pancreatic adenylate cyclase activating peptide or substance P.

16. A method for labeling a peptide with a dye, a therapeutic agent, a chelating moiety or a metal binding site to create a labeled peptide, said method comprising:

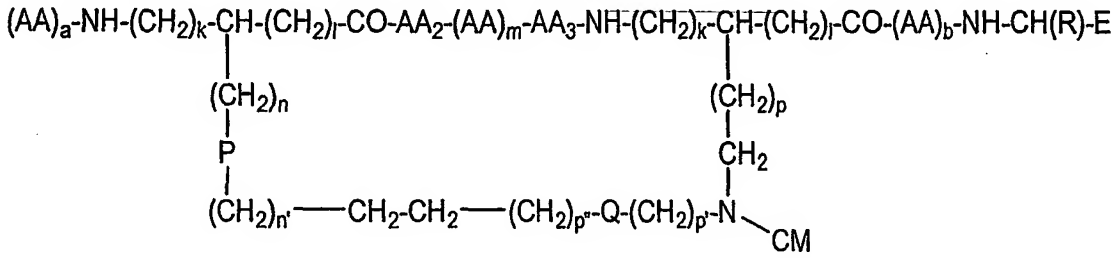
a) synthesizing a macrocyclic ring on said peptide wherein said ring comprises a functional group to which said dye, therapeutic agent, chelating moiety or metal binding site can be attached; and

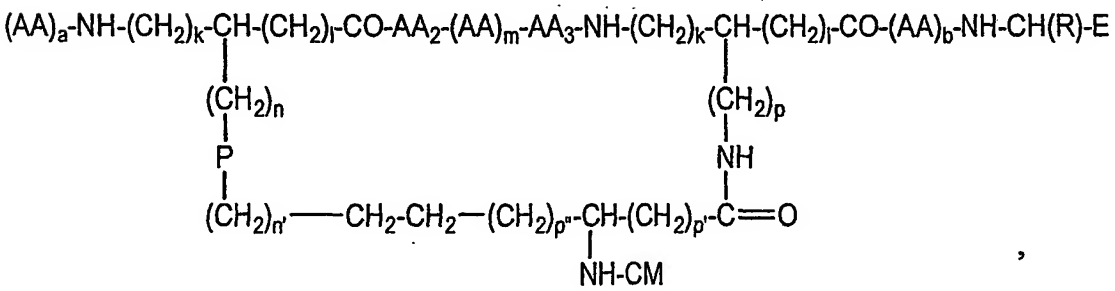
b) attaching said dye, therapeutic agent, chelating moiety or metal binding site to said peptide.

17. The method of claim 16 wherein step (a) is performed using a metathesis reaction.

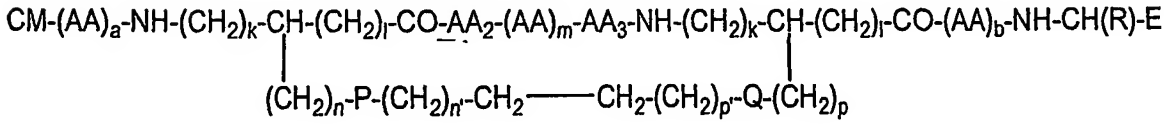
18. The method of claim 17 wherein Grubbs' catalyst is used to catalyze the reaction.

19. The method of claim 16 wherein said labeled peptide is selected from the group consisting of:





and



wherein

AA, $AA_2$, $AA_3$ are natural and unnatural amino acids comprising α-, β- or γ-aminoacids and L- and D- aminoacids;

a, b = 0-10;

k, l = 0-5;

m = 0-20;

n , n' = 1-10;

P, Q is none, O, S, COO, NH-CO, NR, N-CH(=NH)- $NH_2$, NH-CO-NH, NH-COO;

R is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups bearing –OH at any location;

p, p', p'' = 0-10;

E is a group of formula $COOR_4$, $CH_2OR_5$, $CON(R_6)OH$ or $CON(R_7)(R_8)$ wherein

$R_4$ is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups,

$R_5$ is hydrogen or physiologically acceptable, physiologically hydrolyzable ester,

$R_6$ is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups,

$R_7$, $R_8$ is hydrogen or $C_1$-$C_5$ linear or branched chain alkyl groups or taken together form a cyclic alkyl group $C_3$-$C_{10}$; and

CM is a chelating moiety or metal binding site wherein the chelating moiety is labeled with a metal isotope selected from $^{99m}Tc$, $^{203}Pb$, $^{67}Ga$, $^{111}In$, $^{97}Ru$, $^{62}Cu$, $^{64}Cu$, $^{186}Re$, $^{188}Re$, $^{90}Y$, $^{121}Sn$, $^{161}Tb$, $^{153}Sm$, $^{166}Ho$, $^{105}Rh$, $^{177}Lu$ or a radioactive halogen isotope on the understanding that

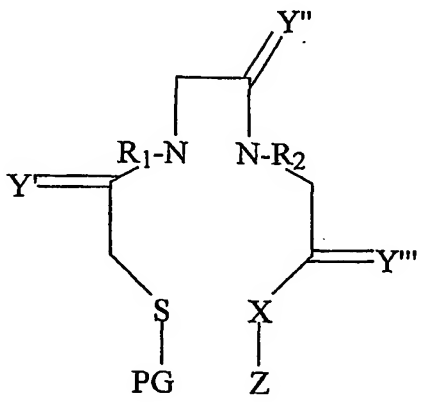
i) if the label is a metal isotope, CM represents a chelating group suitable for the metal and

ii) if the label is a radioactive halogen isotope, the halogen is attached to an aromatic ring,

wherein the CM is attached directly or through a spacing group to the peptide, said CM being attached to the amine through an amide or urea bond or by any other modification which allows attachment of a chelate and which modifications are known to those of skill in the art,

wherein the chelating group is preferably derived from ethylene diamine tetraacetic acid (EDTA), diethylene triamine pentaacetic acid (DTPA), cyclohexyl 1,2-diamine tetraacetic acid (CDTA), ethyleneglycol-O,O'-bis(2-aminoethyl)-N,N,N',N'-diacetic acid (HBED), triethylene tetraamine hexaacetic acid (TTHA), 1,4,7,10-tetraazacyclododecane-N,N',N",N"'-tetraacetic acid (DOTA), 1,4,7-triazacyclononane-

N,N',N''-triacetic acid (NOTA), 1,4,8,11-tetraazacyclotetradecane-N,N',N'',N'''-tetraacetic acid (TETA) or a compound with a general formula




wherein

PG is a sulfur protecting group selected from alkanoyl, arylcarbonyl, arylalkanoyl, acetamidomethyl, tetrahydropyranyl and tetrahydrofuranyl,

Y', Y'', and Y''' are hydrogen or oxygen with the proviso that at least one of them is an O,

$R_1$ and $R_2$ are hydrogen or alkyl ($C_1$-$C_3$),

X = NH or S with the proviso that Y''' is hydrogen when X is S,

Z is PG if X is S, and

Z is hydroxyalkyl, aminoalkyl or carboxyalkyl.

20. The method of claim 16 wherein said peptide is a derivative of: somatostatin, gastrin, gastrin releasing peptide, bombesin, a bombesin antagonist, a gastrin releasing peptide, an adhesion peptide, cholecystokinin, a neurotensin, neuropeptide Y, a vasoactive intestinal peptide, thyroid stimulating hormone, angiotensin, pancreatic adenylate cyclase activating peptide or substance P.

21. The method of claim 16 wherein said dye is selected from fluorescein, fluorescein isothiocyanate, naphthofluorescein, rhodamine derivatives, Texas Red, hydroxycoumarin, indocyanine green, bis-propanoic acid cyanine, acridines, thiazines, phenazines, porphyrins and naphthalimide.

22. The method of claim 16 wherein said therapeutic agent is selected from tamoxifen, adriamycin, phillotoxins, taxol, taxol analogs, bleomycin, doxorubicin, etoposide, methotrexate, vinblastine, vinblastine analogs, dicarbazine and actinomycin D.

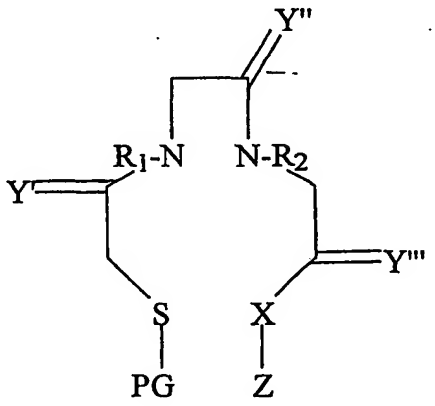
23. The method of claim 16 wherein said chelating moiety or metal binding agent is CM and CM is labeled with a metal isotope selected from $^{99m}Tc$, $^{203}Pb$, $^{67}Ga$, $^{111}In$, $^{97}Ru$, $^{62}Cu$, $^{64}Cu$, $^{186}Re$, $^{188}Re$, $^{90}Y$, $^{121}Sn$, $^{161}Tb$, $^{153}Sm$, $^{166}Ho$, $^{105}Rh$, $^{177}Lu$ or a radioactive halogen isotope on the understanding that

i) if the label is a metal isotope, CM represents a chelating group suitable for the metal and

ii) if the label is a radioactive halogen isotope, the halogen is attached to an aromatic ring,

wherein the CM is attached directly or through a spacing group to the peptide, said CM being attached to the amine through an amide or urea bond or by any other modification which allows attachment of a chelate and which modifications are known to those of skill in the art,

wherein the chelating group is preferably derived from ethylene diamine tetraacetic acid (EDTA), diethylene triamine pentaacetic acid (DTPA), cyclohexyl 1,2-diamine tetraacetic acid (CDTA), ethyleneglycol-O,O'-bis(2-aminoethyl)-N,N,N',N'-diacetic acid (HBED), triethylene tetraamine hexaacetic acid (TTHA), 1,4,7,10-tetraazacyclododecane-N,N',N",N'"-tetraacetic acid (DOTA), 1,4,7-triazacyclononane-N,N',N"-triacetic acid (NOTA), 1,4,8,11-tetraazacyclotetradecane-N,N',N",N'"-tetraacetic acid (TETA) or a compound with a general formula



wherein

PG is a sulfur protecting group selected from alkanoyl, arylcarbonyl, arylalkanoyl, acetamidomethyl, tetrahydropyranyl and tetrahydrofuranyl,

Y', Y", and Y"' are hydrogen or oxygen with the proviso that at least one of them is an O,

$R_1$ and $R_2$ are hydrogen or alkyl ($C_1$-$C_3$),

X = NH or S with the proviso that Y"' is hydrogen when X is S,

Z is PG if X is S, and

Z is hydroxyalkyl, aminoalkyl or carboxyalkyl.

24. A pharmaceutical formulation comprising a peptide of claim 1, claim 6 or claim 11.

25. A method of therapeutically treating an animal, including a person, comprising administering a therapeutic amount of a peptide of claim 1, claim 6 or claim 11 to said animal.

26. A method of diagnosing an animal, including a person, comprising administering a peptide of claim 1, claim 6 or claim 11 to said animal.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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International Bureau

(43) International Publication Date
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PCT

(10) International Publication Number
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(21) International Application Number: PCT/US01/27708

(22) International Filing Date:
7 September 2001 (07.09.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/230,789 7 September 2000 (07.09.2000) US

(71) Applicant *(for all designated States except US)*: **BIOSYNTHEMA INC.** [US/US]; 379 Woodmere Nook Ct., St. Charles, MO 63303 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **SCHMIDT, Michelle, A.** [US/US]; 417 Hickory Manor, Belleville, IL 62223 (US). **ERION, Jack, L.** [US/US]; 379 Woodmere Drive, St. Louis, MO 63303 (US). **SRINIVASAN, Anantachari** [US/US]; 15 Iron Horse Drive #101B, Bedford, NH 03110 (US).

(74) Agent: **EVANS, Lawrence, E.**; Blackwell Sanders Peper Martin LLP, 720 Olive Street, Suite 2400, St. Louis, MO 63101 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
27 December 2002

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

WO 02/020610 A3

(54) Title: CONFORMATIONALLY CONSTRAINED LABELED PEPTIDES FOR IMAGING AND THERAPY

(57) Abstract: Conformational constraints in diagnostic and therapeutic agents in peptides have been introduced by utilization of disulfide bonds and amide cyclizations. These constraints are responsible for altering the stability and specificity of these receptor-targeted agents. Conformationally constrained peptides containing secondary and primary amines, ethers, thioethers, amidines, esters and other functionalities have been synthesized. Methods are disclosed which incorporate multiple features of the above functionalities in the macrocyclic ring of the peptides.

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/27708

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/655 C07K7/06 C07K14/575 A61K38/22 A61K38/08
A61K47/48 A61K49/00 A61K51/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | BASS LA ET AL.: "Identification of the Soluble in Vivo Metabolites of Indium-111-Diethylenetriaminepentaacetic Acid-D-Phe-1-Octreotide" BIOCONJUGATE JOURNAL, vol. 9, 14 February 1998 (1998-02-14), pages 192-200, XP002216302 the whole document | |
| A | WO 99 65508 A (GELLERMAN GARY ;HORNIK VERED (IL); PEPTOR LTD (IL); AFARGAN MICHEL) 23 December 1999 (1999-12-23) the whole document | |
| A | WO 97 09344 A (YISSUM RES DEV CO ;GILON CHAIM (IL); HORNIK VERED (IL); PEPTOR LIM) 13 March 1997 (1997-03-13) page 32 | |

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 10 October 2002 | 29/10/2002 |
| Name and mailing address of the ISA<br>European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Authorized officer<br>Schmidt, H |

Form PCT/ISA/210 (second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 01/27708

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [X] Claims Nos.: 1-26 (all partially)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claim 25 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Although claim 26 is directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.2

Claims Nos.: 1-26 (all partially)

Present claims 1-26 relate to an extremely large number of possible compounds. In fact, the claims contain so many options, variables and possible permutations that a lack of clarity and conciseness within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible.

Furthermore, support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Moreover, the concept of cyclization of peptides to prolong their serum half-life wherein the disulfide bridging group is substituted by a macrocyclic chain and which are highly potent is well known in the prior art (see e.g. WO 99/65508). Hence, it is difficult to determine which parts of the claims may be said to define subject-matter for which protection might legitimately be sought (Article 6 PCT).

Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the peptide derivatives having the amino acid sequences DPhe-Gly-Tyr-DTrp-Lys-Thr-Gly-Thr, DPhe-Gly-Tyr-DTrp-Lys-Thr-Dab-Thr and DAsp-Tyr-Glu-Gly-Trp-Glu-Asp-Phe and their methods.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

# INTERNATIONAL SEARCH REPORT

*Information on patent family members*

rnational Application No
PCT/US 01/27708

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 9965508 A | 23-12-1999 | US 6051554 A | 18-04-2000 |
| | | US 6355613 B1 | 12-03-2002 |
| | | AU 747515 B2 | 16-05-2002 |
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| | | EP 1085896 A1 | 28-03-2001 |
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